

STIC-ILL

ADS

DR/80.247
Adons
20-

From: Prasad, Sarada
Sent: Monday, June 25, 2001 4:57 PM
To: STIC-ILL
Subject: ser no. 09491982

please provide me with the following full text citations--

1. Eur Cytokine Netw 1997, vol.8, pages 51-56. Interleukin-6 receptor antagonists inhibit interleukin-11 biological activity.
2. Neuropharmacology 1994, vol 33, pages 989-94, Fever produced by interelukin-11 (IL-11) injected into the anterior hypothalamic ...
3. Res Immunol. 1998, vol.149, pages 737-40, the interleukin-11/receptor complex: rational design of agoniosts/antagonists and immunoassays potentially usefu; in
4. osteoporosis Int , 1997, vol. 7 suppl 3; pages s47-53. cytokines in the pathogenesis of osteoporosis.

Thank you,

Sarada Prasad, Ph.D.
Patent Examiner
Art Unit 1646
Room CM1-8B17
Tel: 703-305-1009

ADONIS - Electronic Journal Services

Requested by

Adonis

| | |
|---------------------|---|
| Article title | The interleukin-11/receptor complex: Rational design of agonists/antagonists and immunoassays potentially useful in human therapy |
| Article identifier | 0923249498003224 |
| Authors | Jacques_Y Minvielle_S Muller-Newen_G Heinrich_P_C Grotzinger_J Montero-Julian_F Brailly_H Wilkin_J_M Content_J |
| Journal title | Research in Immunology |
| ISSN | 0923-2494 |
| Publisher | Elsevier France |
| Year of publication | 1998 |
| Volume | 149 |
| Issue | 7-8 |
| Supplement | 0 |
| Page range | 737-740 |
| Number of pages | 4 |
| User name | Adonis |
| Cost centre | |
| PCC | \$20.00 |
| Date and time | Tuesday, June 26, 2001 9:49:04 PM |

Copyright © 1991-1999 ADONIS and/or licensors.

The use of this system and its contents is restricted to the terms and conditions laid down in the Journal Delivery and User Agreement. Whilst the information contained on each CD-ROM has been obtained from sources believed to be reliable, no liability shall attach to ADONIS or the publisher in respect of any of its contents or in respect of any use of the system.

The interleukin-11/receptor complex: rational design of agonists/antagonists and immunoassays potentially useful in human therapy

Y. Jacques ⁽¹⁾, S. Minvielle ⁽¹⁾, G. Müller-Newen ⁽²⁾, P.C. Heinrich ⁽²⁾, J. Grötzinger ⁽²⁾,
F. Montero-Julian ⁽³⁾, H. Brailly ⁽³⁾, J.M. Wilkin ⁽⁴⁾ and J. Content ⁽⁴⁾

⁽¹⁾ *Groupe Recherche Cytokines/Recepteurs, Unité INSERM 463, Institut de Biologie, 9 Quai Moncousu, 44035, Nantes Cedex 01 (France),*

⁽²⁾ *Institut für Biochemie, Klinikum der RWTH-Aachen, Pauwelsstrasse 30, D-52057 Aachen (Germany),*

⁽³⁾ *Immunotech-Coulter, 2 Av De Lattre de Tassigny, 13276 Marseille Cedex 9 (France), and*

⁽⁴⁾ *Institut Pasteur (Bruxelles), Department of Virology, Rue Engeland 642, B-1180 Brussels (Belgium)*

Introduction

Interleukin-11 (IL11) is a soluble cytokine initially identified from a non-human primate stromal cell line and characterized on the basis of its growth-promoting effect on an IL6-dependent mouse plasmacytoma cell line (Paul *et al.*, 1990). It has subsequently been shown to be produced and secreted by a variety of mesenchyme-derived cells such as fibroblasts, keratinocytes, chondrocytes, trophoblasts, synoviocytes, bone marrow stromal cells and osteoblasts (reviewed in Du and Williams, 1997).

IL11 was initially described as a growth factor acting at multiple stages of haematopoiesis. *In vitro*, it synergizes with other cytokines (IL3, IL4, steel factor) to support the growth of early, pluripotent progenitors. It has a major role in thrombopoiesis: it stimulates the formation of early megakaryocyte precursors (in combination with IL3) and has a direct effect on the maturation of megakaryocytes by increasing their size and ploidy. IL11 also promotes the formation of erythroid precursors as well as the development of macrophage precursors. It supports survival of B-lymphoid precursors and induces the differentiation of B lymphocytes into Ig-secreting cells, a function which appears to involve the presence of helper T cells. Most of these activities are found when IL11 is injected into mice: there is a general stimulation of medullary haematopoiesis with a more specific enhancement of peripheral

thrombopoiesis and neutropoiesis. Preclinical studies in normal and myelosuppressed animals and more recently phase I and phase II clinical trials in breast cancer patients have demonstrated the potential of IL11 to accelerate multilineage haematopoietic recovery and reduce thrombocytopenia (Tepler *et al.*, 1996).

There is also mounting evidence that IL11 is an anti-inflammatory and protective agent against injury. *In vitro*, it inhibits the production of the inflammatory mediators IL1 β , TNF α , IL12 and nitric oxide (NO) by LPS-activated macrophages, a function which appears to occur through inhibition of the NF- κ B-complex-dependent transcriptional activation (Trepicchio *et al.*, 1997). Through its effect on IL12 production, IL11 can potentially interfere with IL12-dependent Th1-mediated responses (IFN γ production) to infectious agents. IL11 also stimulates the synthesis of type I metalloprotease inhibitors by chondrocytes and synoviocytes, therefore potentially preventing the degradation of cartilage extracellular matrix during articular inflammation. These anti-inflammatory properties of IL11 correlate with its ability to reduce inflammatory symptoms in experimental animal models of acute and chronic inflammatory diseases (LPS-induced systemic inflammation, inflammatory bowel disease, rheumatoid arthritis). In mice treated with combined chemo- and radiotherapy, IL11 prevents apoptosis and accelerates recovery of gastrointestinal and oral mucosal cells damaged as a result of the inflamma-

tory response induced by injury (Du *et al.*, 1994). IL11 is thus considered as a potential therapeutic agent in various inflammatory disorders.

Multiple biological activities for IL11 have also been demonstrated outside the lymphohaematopoietic system. It functions as an adipogenesis inhibitory factor, induces the synthesis of acute phase proteins by hepatocytes, participates in the development of osteoclastic cells, in the differentiation of nerve cells and in spermatogenesis. Recent, IL11 receptor knock-out experiments in the mouse have revealed an essential role for IL11 in female reproduction (Robb *et al.*, 1998).

Many of the activities exhibited by IL11 are displayed by IL6, and to a lesser extent by leukaemia inhibitory factor (LIF), oncostatin M (OSM) and ciliary neurotrophic factor (CNTF). However, IL11 and IL6 also display distinct biological effects. For example, IL6 is an important regulator of T-cell activation and differentiation and has a clear mitogenic activity for a number of lymphoma and myeloma cell lines, two activities which do not seem to be shared by IL11. Conversely, IL11, unlike IL6, stimulates the terminal maturation in the erythroid lineage and stimulates the recovery of small intestinal mucosal cells after cytoablative therapy.

IL11 together with IL6, LIF, OSM and CNTF belongs to the family of long-chain four- α helix bundle cytokines. They also constitute (together with cardiotrophin-1 (CT-1) and more recently sweat gland factor) the subgroup of haemopoietic cytokines which use the gp130 signal transducer for signalling through their high-affinity receptors. Another signal-transducing subunit (gp190) is also shared by LIF, OSM (type I), CNTF and CT-1 receptors, whereas type II OSM receptors use a gp180 transducing subunit. All these transducing subunits (called β chains) are characterized by the existence of large cytoplasmic domains containing consensus boxes and tyrosine residues for binding and recruitment of molecules (tyrosine kinases, transcription factors) responsible for signal transduction. Additional receptor components (α chains) with short cytoplasmic domains have been identified which confer cytokine specificity but do not appear to participate in signal transduction. The IL6R α chain is a membrane-anchored glycoprotein, whereas the CNTFR α chain lacks a cytoplasmic domain and is attached to the membrane through a

glycosylphosphatidyl inositol (GPI) anchor. More recently, an IL11R α chain has been described in the mouse (Hilton *et al.*, 1994) and we have shown the existence of two IL11R α isoforms in humans resulting from alternative exon splicing and most homologous to IL6R α and CNTFR α (Chérel *et al.*, 1995). The first encodes a transmembrane glycoprotein with a short cytoplasmic domain (as is the case of IL6R α) while the second is devoid of a cytoplasmic domain (as in the case of CNTFR α). Both isoforms have similar functional properties when transfected with gp130 in the Ba/F3 cell line, indicating that the cytoplasmic part (32 amino acids) is dispensable for signalling (Lebeau *et al.*, 1997).

All these cytokines use similar signal transduction pathways. LIF, CNTF, CT-1 and OSM induce heterodimerization of gp130 and gp190 (or gp180 in the case of OSM). IL6 induces homodimerization of gp130. In the case of IL11, dimerization of gp130 appears sufficient for signal transduction, although the recruitment of another still unknown receptor subunit has been postulated. This homo/heterodimerization process promotes activation of tyrosine kinases of the janus kinase (Jak/Tyk) family (Lütticken *et al.*, 1994). At present, IL11 has been shown to activate Jak2. However, recent knock-out experiments have demonstrated an obligatory role of Jak1, but not of Jak2, for biological responses elicited by gp130-activating cytokines (Rodig *et al.*, 1998). Activated Jaks phosphorylate tyrosine residues located on the cytoplasmic tails of gp130 and associated subunits. This leads to the docking of additional SH2-containing proteins among which are the signal transducers and activators of transcription (STATs). IL11 has been shown to activate STAT3. Src family tyrosine kinases (Fyn, Yes, Src) can also be mobilized by IL11, as well as the Ras/MAP kinase pathway, phospholipase D, phosphatidylinositol 3 kinase and PKC signalling cascades (Yang and Yin, 1995).

Objectives

Numerous *in vivo* studies have emphasized the potential role of IL6 in several diseases including multiple myeloma, postmenopausal osteoporosis, chronic and immune diseases and AIDS. The molecular cloning of the cytokine and its specific receptor chain has stimulated the generation of agonists or

CNTF = ciliary neurotrophic factor.

CT-1 = cardiotrophin-1.

CTL = cytotoxic T lymphocyte.

GPI = glycosylphosphatidyl inositol.

IL = interleukin.

mAb = monoclonal antibody.

NO = nitric oxide.

OSM = oncostatin M.

PKC = protein kinase C.

STAT = signal transducer, activator of transcription.

TNF = tumour necrosis factor.

antagonists potentially useful in clinical therapy. Less is known concerning the physiopathological implications of IL11, although recent studies have highlighted its role in haematopoietic reconstitution, protection of epithelial mucosal cells, spermatogenesis and female fertility. Also, strategies aimed at designing agonists or antagonists of IL11 action have long been hampered by the lack of knowledge of the specific IL11 receptor chain.

This European project was created to partially fill this gap. Its was initiated on the basis of the identification by one of the participant laboratories of the human receptor for IL11 and the previous and ongoing collaborative expertise of the participating laboratories in the field of IL6, LIF and CNTF. It is a multidisciplinary approach aimed at: (i) modeling the structure of the interleukin-11/receptor complex; (ii) developing immunoassays enabling investigation of the pathological implications of IL-11; and (iii) engineering bioreagents with potential interest in human therapy.

The main steps are: to raise monoclonal antibodies (mAbs) against IL11 and IL11R; to generate mutants of IL11 and IL11R by site-directed mutagenesis; to raise three-dimensional models of IL11, IL11R and their complex based on the structure of other members of the cytokine family; to use antibodies, mutants and molecular modelling predictions in order to define the contact areas and amino acids involved in the interactions between the different partners of the high-affinity IL11 receptor complex, namely IL11 itself, IL11R and gp130 signal transducer; to set up specific immunoassays for the monitoring of IL11, soluble IL11R and soluble gp130 as well as IL11/soluble IL11R and IL11/soluble IL11R/soluble gp130 complexes; to use these immunoassays for the detection of human malignancies associated with abnormalities in IL11 and/or IL11R expression; and to design high-affinity antagonists and agonists (IL11 and IL11R mutants) potentially important for the control and treatment of these malignancies, for example more stable agonists with higher affinity that can be used in the recovery of haematopoietic and intestinal mucosal cell growth following irradiation or chemotherapy in cancer treatment.

Preliminary results

Various constructs have been prepared which link IL11 or IL11R α (ectodomain) to various tags (flag epitope, polyhistidine, thioredoxin, IL2), transfected in different expression systems (*Escherichia coli*, yeast, baculovirus) and the recombinant proteins purified.

The biological activity of these proteins has been evaluated in various cellular systems natu-

rally expressing IL11R and/or gp130 and on transfected cell lines (Ba/F3 system). We confirm that gp130 and IL11R (either as a membrane-anchored or soluble form) are necessary for IL11-mediated biological responses. Activation of gp130 by IL11 involves similar molecular mechanisms as IL6: IL11/IL11R and IL6/IL6R complexes compete for binding to overlapping contact sites on gp130. Both complexes activate STAT3 and STAT1 in a Jak1-dependent manner (Dahmen *et al.*, 1998). Comparison of the IL11 and IL6 efficiencies on transfected and naturally responding cell lines, however, indicate that other component(s) might participate in the structure of the fully active IL11 receptor.

Immunizations have been performed in the mouse with the soluble IL11R α , with synthetic peptides corresponding to different structural regions of the IL11R α ectodomain and by naked DNA technology. Several anti-IL11R α mAbs have been obtained which are being characterized.

A molecular model of IL11 has been built based on the X-ray structure of CNTF (fig. 1). This model allowed prediction of residues in contact with IL11R α and gp130. In analogy with the known three interaction sites of IL6, CNTF and LIF, site I has been predicted to interact with IL11R α and consists of amino acids from the end of the AB loop and the C-terminal part of the D helix. Sites II and III predicted to contact the gp130 homodimer as in the case of IL6 are constituted by residues from the A and C helices (site II) and by residues from the N-terminal half of helix D and the beginning of the AB loop (site III).

Site-directed mutagenesis using a PCR-based method was used to exchange residues predicted to contact IL11R α and gp130. Wild-type IL11 and IL11 mutants were expressed as thioredoxin fusion proteins in *E. coli*. Their binding and functional properties were evaluated in various biological systems. One is the induction of acute phase protein synthesis (α 1-antichymotrypsin, haptoglobin) in HepG2 cells. A second is the proliferation of Ba/F3 cells stably transfected with human gp130 and human IL11R α . This cell line was also used to measure the binding capacities of the IL11 mutants by flow cytometry. Finally, immunoprecipitation and solid-phase ELISA assays were set up to measure the capacity of IL11 mutants to interact with a soluble form of IL11R α and to form a soluble IL11/IL11R α /gp130 ternary complex. Together, the results were in agreement with the predictions made by molecular modelling. Most of the site I mutations resulted in reduced IL11R-binding and biological activity. Some site II or site III mutants exhibited strongly reduced biological activity, while retaining their capacity

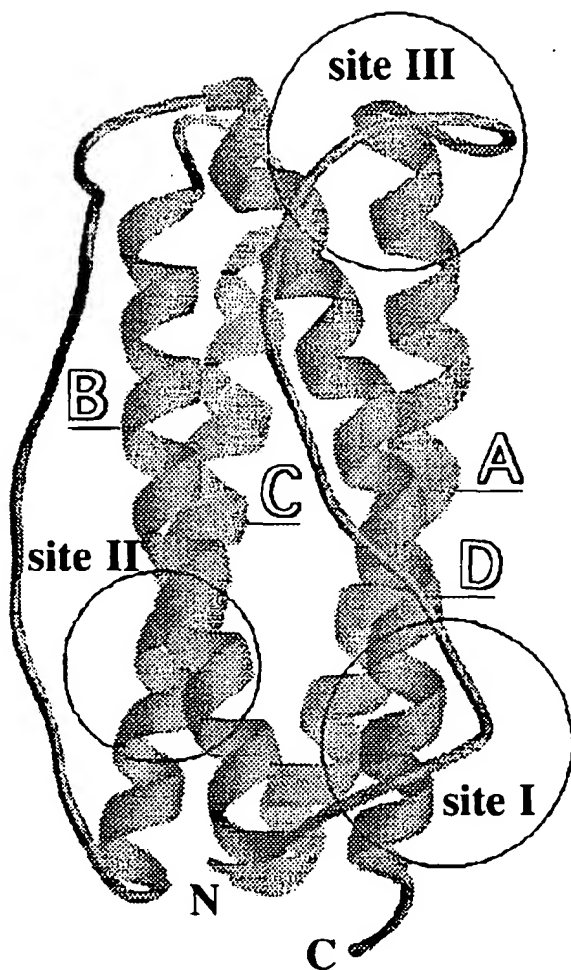


Fig. 1. Ribbon representation of human IL11 obtained by homology-based modelling using human CNTF crystallographic structure as a template.

The alpha helices are designated A,B,C,D. The areas predicted to contact IL11R (site I) and gp130 (sites II and III) are shown.

to interact with IL11R, and represent interesting antagonists of IL11 action.

Key-words: IL11, Haematopoiesis, Receptor; European Biotechnology Program, Review.

References

- Cherel, M., Sorel, M., Lebeau, B., Dubois, S., Moreau, J.F., Minvielle, M. & Jacques, Y. (1995). Molecular cloning of two isoforms of the human receptor for the hematopoietic cytokine Interleukine-11. *Blood*, 86, 2534-2540.
- Dahmen, H., Horsten, U., Kuster, A., Jacques, Y., Minvielle, S., Kerr, I.M., Ciliberto, G., Paonessa, G., Heinrich, P.C. & Muller-Newen, G. (1998). Activation of the signal transducer gp130 by interleukin-11 and interleukin-6 is mediated by similar molecular interactions. *Biochem. J.*, 331, 695-702.
- Du, X.X., Doerschuck, C.M., Orazi, A. & Williams, D.A. (1994). A bone marrow derived growth factor, interleukin-11, stimulates recovery of small intestinal mucosal cells after cytoreductive therapy. *Blood*, 83, 33-37.
- Du, X.X. & Williams, D.A. (1997). Interleukin-11: Review of molecular, cell biology, and clinical use. *Blood*, 89, 3897-3908.
- Hilton, D.J., Hilton, A.A., Raicevic, A., Rakar, S., Harrison-Smith, M., Gough, N.M., Begley, C.G., Metcalf, D., Nicola, N.A. & Willson, T.A. (1994). Cloning of a murine IL-11 receptor alpha-chain: requirement for gp130 for high affinity binding and signal transduction. *EMBO J.*, 13, 4765-4775.
- Lebeau, B., Cherel, M., Montero, F., Müller-Neven, G., Widjenes, J., Godard, A., Minvielle, S. & Jacques, Y. (1997). Reconstitution of two isoforms of the human Interleukin-11 receptor and comparison of their functional properties. *FEBS Lett.*, 407, 141-147.
- Lütticken, C., Wegenka, U.M., Yuan, J., Buschmann, J., Schindler, C., Ziemiecki, A., Harpur, A.G., Wilks, A.F., Yasukawa, K., Taga, T. & al (1994). Association of transcription factor APRF and protein kinase Jak1 with the interleukin-6 signal transducer gp130. *Science*, 263, 89-92.
- Paul, S.R., Bennett, F., Calvetti, J.A., Kelleher, K., Wood, C.R., O'Hara, R.M.Jr., Leary, A.C., Sibley, B., Clark, S.C., Williams, D.A. & Yang, Y.C. (1990). Molecular cloning of a cDNA encoding interleukin 11, a stromal cell-derived lymphopoietic and hematopoietic cytokine. *Proc Natl Acad Sci USA*, 87, 7512-7516.
- Robb, L., Li, R., Hartley, L., Nandukkar, H.H., Kocntgen, F. & Begley, C.G. (1998). Intertility in female mice lacking the receptor for interleukin-11 is due to a defective uterine response to implantation. *Nature Med.*, 4, 303-308.
- Rodig, S.J., Meraz, M.A., White, J.M., Lampe, P.A., Riley, J.K., Arthur, C.D., King, K.L., Sheehan, K.C.F., Yin, L., Pennica, D., Johnson, E.M.Jr. & Schreiber, R.D. (1998). Disruption of the Jak1 gene demonstrates obligatory and nonredundant roles of the Jaks in cytokine-induced biological responses. *Cell*, 93, 373-383.
- Tepler, I., Elias, L., Smith, W.H., Hussein, M., Rosen, G., Chang, A.Y.-C., Moore, J.O., Gordon, M.S., Kuca, B., Beach, K.J., Loewy, J.W., Garnick, M.B. & Kaye J.A. (1996). A randomized placebo-controlled trial of recombinant human interleukin-11 in cancer patients with severe thrombocytopenia due to chemotherapy. *Blood*, 87, 3607-3614.
- Trepicchio, W.L., Wang, L., Bozza, M. & Dorner, A.J. (1997). IL-11 regulates macrophage effector function through the inhibition of nuclear factor- κ B. *J. Immunol.*, 159, 5661-5670.
- Yang, Y.C. & Yin, T. (1995). Interleukin (IL)-11-mediated signal transduction. *Ann. N. Y. Acad. Sci.*, 762, 31-40.